No. 41.

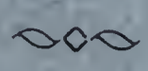
THE CONSTITUTION OF

EPINEPHRINE

BY

H. A. D. JOWETT, D.Sc.

(From the Transactions of the Chemical Society, 1904)



THE WELLCOME CHEMICAL RESEARCH LABORATORIES

FREDERICK B. POWER, PH.D., *Director*

6, King Street, Snow Hill

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XXIV.—*The Constitution of Epinephrine.*

By Hooper Albert Dickinson Jowett.

"Epinephrin," the name given by Abel and Crawford to the active principle of the suprarenal gland, was first isolated by them in an impure condition in 1897 (Johns Hopkins Hospital Bulletin, No. 76), and a similar substance, also more or less impure, but prepared by a different method, was obtained by von Fürth (*Zeit. physiol. Chem.*, 1900, 29, 105), who called it "suprarenin."

In 1901, the active principle was isolated in a crystalline condition by Takamine (*Amer. J. Pharm.*, 1901, 73, 523) and called by him "adrenalin," and shortly afterwards it was prepared by a different method by Aldrich (*Amer. J. Physiol.*, 1901, 5, 457).

The three names "epinephrin," "suprarenin," and "adrenalin," therefore refer to the same substance, although Abel (*Ber.*, 1903, 36, 1839) has since adopted the term "epinephrin hydrate." As this author was the first to isolate the substance, although in an impure condition, it would seem that the name originally assigned by Abel to the active principle should be the one adopted. Takamine, from the results of analyses of crystalline epinephrine, proposed the formula

$C_{10}H_{15}O_3N$, and showed that, although it acted as a mono-acidic base, it gave no reaction with the usual alkaloidal reagents. Aldrich, however, preferred the slightly different formula $C_9H_{13}O_3N$.

Abel, who carefully purified his material, adopted the formula $C_{10}H_{13}O_3N,\frac{1}{2}H_2O$, but adduced absolutely no evidence to show that it contained any water of crystallisation. His results agree equally well with Aldrich's formula $C_9H_{13}O_3N$. This is seen from the following numbers calculated for each formula:

(Aldrich's formula) $C_9H_{13}O_3N$ requires C = 59·0; H = 7·1; N = 7·6.
(Abel's formula) $C_{10}H_{13}O_3N,\frac{1}{2}H_2O$ requires C = 58·8; H = 6·9; N = 6·9.
Abel actually found C = 58·4 to 58·7; H = 6·8 to 7·2; N = 7·1 to 7·6 per cent.

Von Fürth (*Monatsh.*, 1903, 24, 261) confirmed the formula $C_9H_{13}O_3N$ by analyses and molecular weight determinations.

Pauly (*Ber.*, 1903, 36, 2945), from the results of the analysis of very carefully purified material, also confirmed this formula, so that it must be considered the most probable of those proposed. No crystalline salts or derivatives have been described, but von Fürth prepared a tribenzoyl- and a tribenzenesulpho-derivative. He also showed that it contained no methoxyl group, and that it yielded methylamine by treatment with concentrated acids.

Of the degradation products of epinephrine, only protocatechuic acid, formed by fusion with potassium hydroxide, has been positively identified, although substances giving the pyrrole, skatole, or catechol reactions have been obtained by different observers. Von Fürth suggested for the base the partially developed formula $[CH_3\cdot NC_2H\cdot OH]C_6H_6(OH)_2$, and Pauly, who determined its specific rotation, suggested that it contained a hydroxylated benzene residue attached to one of five possible complexes, of which the most probable were the following:

$$\begin{array}{l}\cdot CH\cdot OH\\ |\\ CH_2\cdot NH\cdot CH_3\end{array} \quad \text{and} \quad \begin{array}{l}\cdot CH\cdot NH\cdot CH_3\\ |\\ CH_2\cdot OH\end{array}.$$

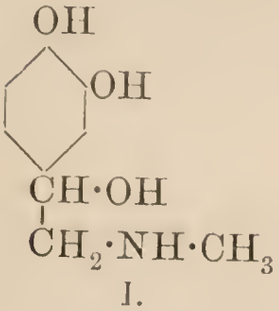
In this way, the formation of catechol, skatole, or pyrrole derivatives would be easily explained.

In the present investigation, I have confirmed the formula $C_9H_{13}O_3N$, first proposed by Aldrich, by analyses of carefully purified material and by molecular weight determinations. By fusion with potassium hydroxide, a small quantity of a crystalline substance giving the reactions of protocatechuic acid was isolated, but the amount was so small that it was doubtful whether the presence of this complex in the original substance could be correctly deduced from its formation.

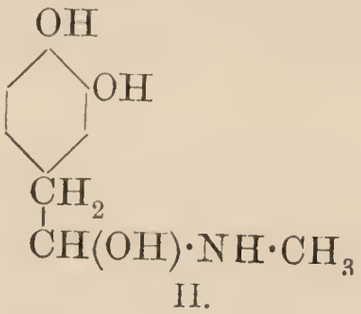
By oxidation with permanganate, oxalic and formic acids and methylamine were obtained.

By methylation and subsequent oxidation with permanganate, trimethylamine and veratric acid were obtained, thus proving the existence of the complexes $C_6H_3(OH)_2 \cdot C$ and $NH(CH_3)$ in the original base.

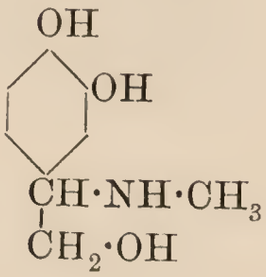
From these results, the following constitutional formulæ of epinephrine may be deduced:



I.



II.



III.

Of these formulæ, I is the more probable, for if II were correct, then, after methylation and subsequent oxidation, we should expect the product to yield homoveratric acid, $C_6H_3(OH)_2 \cdot CH_2 \cdot CO_2H$, whereas veratric acid was actually obtained, whilst III would not so readily explain the formation of pyrrole or skatole derivatives. Formula I may therefore be considered to represent correctly the constitution of epinephrine, and it serves to explain the ordinary reactions of the base as well as the formation of pyrrole, skatole, or catechol derivatives as degradation products.

Experimental.

The Formula and Properties of Epinephrine.

The crude crystalline material was first freed from inorganic impurities by the method previously adopted by other observers, namely, solution of a salt in alcohol and fractional precipitation with ether. It was finally purified by fractional precipitation of the base from the aqueous solution of its hydrochloride by ammonia.

The analyses of four different specimens were sufficient to confirm the formula $C_9H_{13}O_3N$.

0·1184 gave 0·2546 CO_2 and 0·0798 H_2O. C = 58·6 ; H = 7·5.
0·1082 „ 0·2306 CO_2 „ 0·0720 H_2O. C = 58·1 ; H = 7·4.
0·1238 „ 0·2642 CO_2 „ 0·0816 H_2O. C = 58·2 ; H = 7·3.
0·0990 „ 0·2136 CO_2 „ 0·0644 H_2O. C = 58·8 ; H = 7·2.
0·1342 „ 9·2 c.c. N at 15° and 755 mm. N = 7·8.
0·0946 in 24·78 glacial acetic acid gave Δt −0·11°. M. W. = 135.
$C_9H_{13}O_3N$ requires C = 59·0 ; H = 7·1 ; N = 7·6 per cent. M. W. = 183.

The determination of the specific rotation of the base in dilute acetic acid solution gave the following result:

$\alpha_D = -10'$; $l = 0{\cdot}25$ dcm.; $c = 2{\cdot}084$; $[\alpha]_D = -32{\cdot}0°$.

Pauly (*loc. cit.*) found $[\alpha]_D - 43°$, but considering the small observed angle (10′) the above figures do not vary beyond the limits of experimental error.

The general statements of previous observers as to the solubility of epinephrine in various solvents and its behaviour towards alkaloidal reagents were confirmed. The base did not react with phenylhydrazine.

Oxidation with Permanganate.

Five grams of epinephrine were dissolved in dilute sulphuric acid and oxidised at the ordinary temperature with a 1 per cent. solution of permanganate, 30 grams of this reagent being required to produce a permanent colour. The product yielded methylamine, which was identified by its platinichloride.

0·1646 gave 0·0688 Pt. Pt = 41·8.

$(CH_5N)_2,H_2PtCl_6$ requires Pt = 41·3 per cent.

The acids obtained were formic and oxalic acids.

Fusion with Potassium Hydroxide.

Five grams of epinephrine were added to 25 grams of potassium hydroxide and the mass fused at as low a temperature as possible. The melt was then dissolved in water, acidified, and extracted with ether. The residue, after distilling off the ether, was obtained crystalline and gave the characteristic protocatechuic acid reaction on adding successively ferric chloride and sodium carbonate. The amount obtained was insufficient to admit of further examination.

Methylation and Subsequent Oxidation with Permanganate.

Four grams of epinephrine were dissolved in 50 c.c. of methyl alcohol in which 1 gram of sodium had been dissolved, and 8 grams of methyl iodide added. The mixture was then heated in a sealed tube at 100° for four hours, the alcohol distilled off, and the operation repeated with the residue. After the second methylation, the residue was dissolved in water and then added to an aqueous solution of 17 grams of silver nitrate, the silver iodide quickly filtered off, and the filtrate saturated with hydrogen sulphide and again filtered. The filtrate was then oxidised with a 2 per cent. solution of perman-

ganate at the ordinary temperature, when 10 grams were required to produce a permanent colour. The product, when worked up in the usual way, yielded a volatile base which was identified as trimethylamine.

0·1588 Pt salt gave 0·059 Pt. Pt = 37·1.

$(C_3H_9N)_2,H_2PtCl_6$ requires Pt = 37·1 per cent.

The crystalline acid obtained from the ethereal extract was recrystallised from hot water until its melting point was constant; it formed white, acicular crystals, sparingly soluble in cold water and melting sharply at 179°. The aqueous solution gave no reaction with ferric chloride.

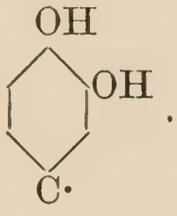
0·1128, dried at 110—120°, gave 0·2446 CO_2 and 0·057 H_2O. C = 59·1 H = 5·6.

$C_9H_{10}O_4$ requires C = 59·3; H = 5·5 per cent.

This compound was therefore identified as veratric acid.

The Constitution of Epinephrine.

Since the product obtained by exhaustive methylation yields veratric acid by oxidation with permanganate, epinephrine must contain the complex :



As it yields trimethylamine by the foregoing treatment and methylamine by oxidation of the original base, it must therefore contain the group ·NH(CH$_3$), which, being split off by simple oxidation, is most probably attached to the side-chain and not to the benzene nucleus.

As epinephrine yields tribenzoyl- and tribenzenesulpho-derivatives, it probably contains three hydroxyl groups, of which two are attached to the benzene nucleus.

The only probable formulæ which would comply with the above conditions and also conform to Pauly's hypothesis (*loc. cit.*) are those indicated on p. 194.

Of these, I is the most probable, for if II were correct we should expect after methylation and subsequent oxidation to obtain homoveratric acid and not veratric acid. Moreover, III would not so readily explain the formation of pyrrole or skatole derivatives.

Formula I is therefore the most probable formula for epinephrine, as by it the formation of catechol or protocatechuic acid is easily explained

as well as the reducing properties of the base. It also contains one of the two side-chains regarded as probable by Pauly (*loc. cit.*). By the union of the nitrogen atom to the benzene ring, pyrrole derivatives would be formed, as has been stated to be the case.

Addendum.—Since this paper was written, a communication by Abel has appeared (*Ber.*, 1904, 37, 368) in which he still adheres to the formula $C_{10}H_{13}O_3N,\frac{1}{2}H_2O$. He states, however (*loc. cit.*, 381), that 0·2288 gram of the base, when heated for one hour at 145° and subsequently for one hour at 155—160° in a vacuum, lost only 0·0014 gram or 0·6 per cent. This experiment apparently only affords further evidence that the base contains no water of crystallisation.

THE WELLCOME CHEMICAL RESEARCH LABORATORIES,
LONDON, E.C.

R. CLAY AND SONS LTD BREAD STREET HILL, E C., AND BUNGAY, SUFFOLK.